INTRODUCTION

Feline calicivirus (FCV) is an important pathogen of cats. Infection with FCV can result in a wide range of clinical diseases, the most significant of which is upper respiratory tract disease (Dawson et al., 1994). Other diseases associated with FCV infection include oral ulceration, acute arthritis (Dawson et al., 1994), jaundice and death from infection in utero (Ellis, 1981). A carrier state is common after FCV infection and many cats continue to produce virus for a number of years in the absence of any symptoms of disease (Wardley, 1976). Highly virulent strains of FCV have been described in the USA (Hurley et al., 2004; Pedersen et al., 2000; Schorr-Evans et al., 2003) and more recently in the UK (Coyne et al., 2006). Symptoms associated with infection with these strains included pyrexia, anorexia and jaundice and resulted in a high mortality rate. Additional symptoms of the US strains included skin oedema and ulceration; these were not observed in the UK outbreak.

FCV, a member of the genus Vesivirus of the family Caliciviridae, is a non-enveloped virus with a 7.7 kb positive-sense RNA genome (Carter et al., 1992) covalently linked to a viral protein, VPg (Burroughs & Brown, 1978; Herbert et al., 1997). The genome encodes three open reading frames (ORFs). ORF1 encodes a polyprotein that is co-translationally cleaved to generate the non-structural proteins, which include a 2C picornavirus-like helicase, a 3C-like protease and a 3D-like polymerase (Neill, 1990; Sosnovtseva et al., 1999). ORF2 and -3 are expressed from a VPg-linked subgenomic RNA species. ORF2 encodes the capsid protein precursor and ORF3 encodes a small protein recently shown to be a minor virion component that is possibly involved in RNA encapsidation (Sosnovtsev & Green, 2000).

FCV shows a restricted tropism for feline cells, although non-permissive, non-feline cells can support virus replication following transfection of FCV RNA. This suggests that the cell tropism results from either virus binding or virus entry. Recently Makino et al. (2006) isolated a functional receptor for a number of strains of FCV – this was identified as junctional adhesion molecule-A (JAM-A). JAM-A is a member of the immunoglobulin superfamily. It is thought to be involved in apical tight junction assembly and to mediate leukocyte trafficking. JAM-A is expressed on a number of cells including epithelial cells, endothelial cells, leukocytes, platelets and red blood cells (Mandell & Parkos, 2005). FCV infection usually results in infection of the respiratory tract; the widespread expression of JAM-A suggests that additional factors are required for respiratory tissue specificity. Makino et al. (2006) showed that anti-JAM-A antibodies did not completely abolish FCV binding to cells, also suggesting that other factors may be involved.

Sialic acids form a family of negatively charged sugar molecules usually found at the ends of oligosaccharides, attached to glycoproteins, glycolipids and proteoglycans. Sialic acid is normally linked to galactose at the terminus of the oligosaccharide by α2,3 or α2,6 bonds or to an internal sialic acid by α2,8 bonds. A number of viruses, including both enveloped and non-enveloped RNA and DNA viruses, have been shown to use sialic acids as a component of their cellular receptor. These include members of the Orthomyxoviridae (Suzuki et al., 2000), Paramyxoviridae (Suzuki et al., 2001), Picornaviridae (Alexander & Dimock, 2002; Shah & Lipton, 2002; Stevenson et al., 2004; Stoner et al., 1973; Uncapher et al., 1991; Utagawa et al., 1982; Zhou et al., 2000), Paroviridae (Barbis et al., 1992; Kaludov et al., 2001; Walters et al., 2001), Coronaviridae (Schulz et al., 2001).
1996; Winter et al., 2006), Papovaviridae (Dugan et al., 2005; Fried et al., 1981; Liu et al., 1998), Reoviridae (Isa et al., 2006; Barton et al., 2001a) and Adenoviridae (Arnberg et al., 2000). Some viruses bind preferentially to sialic acid via a specific glycosidic linkage (Arnberg et al., 2000; Blackburn et al., 2005; Dugan et al., 2005; Helander et al., 2003; Kaludov et al., 2001; Liu et al., 1998; Rogers & Paulson, 1983; Stevenson et al., 2004) and this may lead to restrictions in host range, tissue tropism and pathogenesis. An example of this can be seen by the preference of avian influenza for sialic acid and growth of this virus in intestinal cells in birds, whilst human influenza uses sialic acid and grows in cells of the respiratory tract (Couceiro et al., 1993; Matrosovich et al., 2004).

We examined the role of sialic acid in FCV infection of feline cells using a number of techniques involving metabolic inhibitors, proteases, lectins and a linkage-specific neuraminidase, and using equine Influenza A virus (EIV) as a control virus known to bind to sialic acid. Our data indicated that FCV binds to an sialic acid present on an N-linked glycoprotein.

### METHODS

#### Cells and virus.
Crandall–Reese feline kidney (CRFK), AK-D, BHK, Vero, 293T and MDCK cells were obtained from ECACC. Cells were grown in Glasgow’s minimal essential medium (GMEM) supplemented with 10% fetal calf serum, 100 U penicillin ml⁻¹ and 100 µg streptomycin ml⁻¹. The F9 strain of FCV was kindly provided by Dr Chris Helps, School of Clinical Veterinary Science, University of Bristol, UK. EIV strain Sussex was kindly provided by Dr Janet Daly, Animal Health Trust, Newmarket, UK.

#### Reagents and antibodies.
Sodium periodate (Fluka), trypsin, chymotrypsin, Pronase, proteinase K (all from Sigma), N-acetyl neuraminic acid (Calbiochem), N-glycolyl neuraminic acid, sialylactose (SL; Calbiochem) and galactose (Sigma) were all dissolved in water. Sodium periodate (Fluka), trypsin, chymotrypsin, proteinase K (all from Sigma) were dissolved in PBS supplemented with 1 mM CaCl₂ and 0.5 mM MgCl₂. Other reagents included Vibrio cholerae neuraminidase (Calbiochem), Streptococcus pneumoniae sialidase S (Glyko), anti-capsid monoclonal antibody IgG (Novoceastra), anti-influenza virus nucleoprotein polyclonal antibody, Alexa Fluor 488 anti-rabbit antibody (Molecular Probes) and Alexa Fluor 488 anti-mouse antibody (Molecular Probes).

#### Labelling of F9 with 35S methionine/cysteine and purification of F9 virions.
F9 cells were treated with 2 mM benzylGalNAc in GMEM for 3 days, 2 µg tunicamycin ml⁻¹ in GMEM for 24 h or 16 h at 37°C. Virus was harvested by freeze–thawing the infected cells. Cell debris was removed by centrifugation (11 000 g for 15 min).

Virus was purified using the method described by Zhou et al. (1994). Briefly, virus was precipitated using 0.2 M NaCl and 10% PEG 3350. Pelleted virus was resuspended in 200 mM boric acid (pH 7.4) containing 0.5 M NaCl. Virions were isolated by isopycnic CsCl gradient centrifugation (1.31 g ml⁻¹) for 20 h at 194 000 g in a Beckman SW55Ti rotor. Fractions containing virus were subjected to further ultracentrifugation to concentrate the samples and remove the CsCl.

#### Binding of 35S methionine/cysteine-labelled F9 virus to various cell lines.
CRFK, AK-D, BHK, Vero, 293T and MDCK cells (4 × 10⁵) were plated into 96-well plates. Purified 35S methionine/cysteine-labelled F9 (50 000 c.p.m.) was incubated with the cells for 45 min on ice. Cells were then washed three times with ice-cold PBS. Cells were lysed with 0.1% SDS and 0.1 M NaOH. Total radioactivity in the cell lysate was determined by liquid scintillation counting. Control samples were included where the virus was incubated with a polyclonal anti-FCV capsid antibody for 1 h on ice before incubation with the cells.

#### Infectivity assays.
Cells (10⁶) were plated into 24-well plates with 13 mm diameter glass covers. After incubation overnight at 37°C, cells were treated with various inhibitors or enzymes as described below. The treated cells were infected with FCV or EIV strain Sussex as a control at an m.o.i. of 10 and incubated at 37°C for 30 min. Cells were washed three times with PBS and overlaid with growth medium. Plates were incubated for 6 h at 37°C before being fixed with 4% formaldehyde in PBS. Samples were then analysed by immunofluorescence as described below.

#### Immunofluorescence.
Fixed cells were permeabilized by the addition of 0.2% Triton X-100 and incubated for 5 min at room temperature. Cells were then washed twice with PBS containing 0.1% newborn calf serum (PBS-NCS). IgG anti-FCV capsid monoclonal antibody was added at a dilution of 1:1000, or rabbit anti-influenza A virus nucleoprotein (NP) polyclonal antibody (kindly provided by Dr Paul Digard, University of Cambridge, UK) was added at a dilution of 1:500. Plates were incubated at room temperature for 30 min. Cells were then washed twice with PBS-NCS and the secondary antibody (diluted 1:1000) and DAPI were added and incubated for a further 30 min. Samples were washed three times with PBS-NCS, the coverslips were removed and samples were mounted onto glass slides using ProLong Gold antifade mountant (Molecular Probes). Samples were examined using a Leica SP confocal microscope and TEC NT software. Laser and microscope settings were according to the manufacturer’s instructions.

#### Treatment of cells with sodium periodate.
After incubation overnight at 37°C, cells were treated with 0.1 or 1 mM sodium periodate (NaIO₄) for 30 min at 4°C. Residual NaIO₄ was removed by reaction with an equal volume of 1% glycerol. Binding and infectivity assays were carried out as described above.

#### Treatment of cells with neuraminidases.
After incubation overnight at 37°C, cells were treated with 100 mU neuraminidase ml⁻¹ (from V. cholerae or Streptococcus pneumoniae) for 60 min. Cells were washed three times with PBS, and binding and infectivity assays were carried out as described above.

#### Treatment of cells with proteases.
After incubation overnight at 37°C, cells were treated with 0.1–0.5 mg trypsin or chymotrypsin ml⁻¹ for 30 min at 37°C. Cells were washed three times with PBS, and binding and infectivity assays were carried out as described above. Treated, uninfected cells were used as control samples and counted to confirm that the treatment did not affect the number of input cells.

#### Treatment of cells with metabolic inhibitors.
After incubation overnight at 37°C, cells were treated with 2 mM benzylGalNAc in GMEM for 3 days, 2 µg tunicamycin ml⁻¹ in GMEM for 24 h or...
25 μM PDMP in GMEM supplemented with 10% fetal calf serum for 3 days. Cells were washed three times with PBS, and binding and infectivity assays were carried out as described above.

**Treatment of cells with peptide N-glycanase (PNGase F).** After incubation overnight at 37°C, cells were treated with 100 U PNGase F (Calbiochem) ml⁻¹ for 60 min at 37°C. Cells were then washed three times with PBS, and binding and infectivity assays were carried out as described above.

**Inhibition of binding by lectins.** After incubation overnight at 37°C, cells were incubated with M. amurensis lectin, Sambucus nigra lectin, wheatgerm agglutinin or concanavalin A (100 μg ml⁻¹) for 60 min at 4°C. Cells were washed, and binding and infectivity assays were carried out as above.

**Inhibition of binding by oligosaccharides.** F9 strain of FCV was incubated with 20–120 mM N-acetyl neuraminic acid (NANA), N-glycolyl neuraminic acid (NGNA), galactose or SL for 60 min at 4°C. The virus was then used in binding and infectivity assays as described above.

**RESULTS**

**FCV binds to and infects cells of feline origin only**

We examined the ability of FCV strain F9 to bind to cells of feline, canine, murine, simian and human origin. As shown in Fig. 1(a), we demonstrated that F9 bound most efficiently to cells of feline origin (CRFK and AK-D). The virus bound less well to canine cells (MDCK) (40–50% of that bound to feline cells) and negligibly to murine (BHK), simian (Vero) and human (293T) cells. F9 readily infected both feline cell lines, resulting in similar levels of cytopathic effect (CPE); in contrast, the canine, murine, simian and human cells were non-permissive for FCV infection (Fig. 1b).

**FCV binding and infection requires carbohydrate moieties**

Kreutz et al. (1994) reported that FCV interacts with oligosaccharides on the cell surface. We tested this by pre-treating the cells with 0.1 or 1 mM NaIO₄ before binding or infection with FCV. NaIO₄ destroys carbohydrate groups without altering proteins or membranes by oxidation of neighbouring hydroxyl groups on sugars to dialdehydes at acidic pH (Martinez-Barragan & del Angel, 2001; Woodward et al., 1985). As shown in Fig. 2(a), treatment of cells with 0.1 or 1 mM NaIO₄ reduced binding of F9 to CRFK cells to 54 and 22%, respectively, compared with untreated cells. Fig. 2(b) shows the results of infection of periodate-treated cells with F9 and other clinical isolates of FCV. The clinical isolates were inhibited by periodate treatment to a similar degree to that seen with F9. F9 infection was reduced to 48% for 0.1 mM and 21% for 1 mM periodate, whilst the inhibition seen for the clinical isolates ranged from 41 to 52% for 0.1 mM and from 16 to 33% for 1 mM periodate. As a control for a virus known to use sialic acid-containing carbohydrates, we examined the effect of periodate treatment on the infection of MDCK cells by EIV strain Sussex. EIV infection was reduced to 47% for 0.1 mM and 23% for 1 mM.

**FCV binding/infection requires sialic acid**

In order to investigate the nature of the oligosaccharides involved in the interaction between FCV and CRFK cells, we treated cells with 100 mU V. cholerae neuraminidase ml⁻¹, which cleaves α₂,3-linked and α₂,6-linked terminal sialic acid residues and α₂,8-linked internal sialic acid residues. Pre-treatment of cells with neuraminidase reduced F9 binding to 23% compared with the untreated control (Fig. 3a). Infection of CRFK cells by F9 and the clinical isolates was also inhibited by V. cholerae neuraminidase. F9 infection was reduced to 23%, whilst infection by the clinical isolates was reduced to 22–31%. As expected, EIV infection was also inhibited by V. cholerae neuraminidase, with the number of virus-positive cells being reduced to 35%. We also examined the specificity of the linkage required for the interaction between FCV and sialic acid using sialidase S (from Streptococcus pneumoniae), which

![Fig. 1. FCV strain F9 binds to and infects cells of feline origin only. (a) [³⁵S]Methionine/ cysteine-labelled virus (50 000 c.p.m.) was bound to each of the cell lines indicated. Binding of radiolabelled virus was detected by scintillation counting. (b) Tenfold serial dilutions of F9 were used to infect each of the cell lines in six-well plates. CPE was detected by fixation of cell monolayers with 4% formaldehyde in PBS and staining with 0.1% toluidine blue. These experiments were repeated four times and one representative set of results is shown.](http://vir.sgmjournals.org)
cleaves α2,3-linked sialic acid only. Pre-treatment of cells with this neuraminidase had no effect on F9 binding or FCV infection (Fig. 3a and b, respectively). However, EIV infection was inhibited by sialidase S, with infection being reduced to 34 %.

**FCV binds to α2,6-linked sialic acid**

Additional experiments were carried out to identify the sialic acid linkage required for FCV infection. We used two lectins to examine this: *M. amurensis* lectin (MAL), which
binds preferentially to α2,3-linked sialic acid, and *Sambucus nigra* lectin (SNL), which binds preferentially to α2,6-linked sialic acid. The results shown in Fig. 4(a) demonstrated that pre-incubation of cells with SNL inhibited F9 binding, reducing binding to 25% that of untreated cells, but pre-incubation of cells with MAL had no effect on binding. SNL was also able to inhibit infection of CRFK cells by F9 and all of the clinical isolates of FCV as shown in Fig. 4(b). F9 infection was reduced to 22% and infection by the clinical isolates to 21–31%. MAL had no effect on infection of cells by any of the strains of FCV, but was able to inhibit infection of MDCK cells by EIV, reducing infection to 21%. MAL or SNL have not been shown to be mitogenic; however, control samples were included to confirm that lectin treatment did not affect the number of input cells. Neither lectin had any effect on the number of input cells (data not shown).

**FCV binds to an N-linked glycoprotein containing sialic acid**

We used the metabolic inhibitor PDMP to examine whether the sialic acid moiety was attached to a glycolipid. The data shown in Fig. 5(a, b) demonstrated that PDMP treatment of CRFK cells had no effect on F9 binding or FCV infection, respectively. The lack of inhibition by PDMP suggested that the sialic acid-containing glycan important for FCV infection was not linked to a glycolipid. However, infection with the control virus, EIV, was inhibited by PDMP treatment, reducing infection to 55%. This suggested that glycolipid moieties might be involved in influenza virus infection. In order to investigate further the nature of the receptor, we carried out a series of experiments using the proteases chymotrypsin and trypsin. As shown in Fig. 5(a), chymotrypsin treatment of cells reduced binding of F9 to 5%, whilst trypsin treatment reduced binding to 42% of that of the untreated control. We also examined the ability of F9 and the clinical isolates of FCV to infect cells pre-treated with the proteases. Fig. 5(b) shows that treatment of cells with chymotrypsin almost completely inhibited infection (2–5% virus-positive cells were observed), whilst trypsin treatment again only partially inhibited infection, reducing infection to 21–29%. EIV infection was also inhibited by proteases, with infection reduced to 31% for chymotrypsin and 35% for trypsin. Control samples of treated, uninfected cells were included to confirm that this treatment did not affect the number of input cells. The concentrations of chymotrypsin and trypsin used in these assays did not result in cells detaching from the monolayer, as the number of cells was similar in mock- and protease-digested cells; PDMP treatment also had no effect on the number of input cells (data not shown).

In order to establish whether the sialic acid-containing glycan was attached by O-linked or N-linked glycosylation, we treated cells with tunicamycin (which inhibits N-linked glycosylation), PNGase F (which cleaves N-linked glycans) or benzylGalNAc (which inhibits O-linked glycosylation). As shown in Fig. 6(a), treatment of cells with tunicamycin or PNGaseF reduced F9 binding to 41 and 38% of the control, respectively. BenzylGalNAc treatment of cells had no effect on F9 binding. Fig. 6(b) shows that inhibition or removal of N-linked glycans by tunicamycin and PNGase F also inhibited virus infection. F9 infection was reduced to 41% in tunicamycin-treated cells and 44% in PNGase F-treated cells. Infection by the FCV clinical isolates was also inhibited (36–41% for tunicamycin-treated cells and 38–42% for PNGase F-treated cells). EIV infection was reduced to 28% in tunicamycin-treated cells and 26% in untreated cells.

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**Fig. 4.** FCV interacts with α2,6-linked sialic acid. CRFK cells were incubated with 100 μg MAL or SNL ml⁻¹. (a) Radiolabelled virus was bound to lectin-treated cells and virus binding was detected by scintillation counting. (b) Lectin-treated cells were infected with F9, FCV clinical isolates or EIV (m.o.i. = 10). Virus infection was detected by indirect immunofluorescence. These experiments were repeated four times and one representative set of results is shown.
PNGase F treated cells. BenzylGalNAc treatment of cells had no effect on FCV or EIV infection. Each of the treatments was checked with control samples of treated, uninfected cells, which were counted to confirm that the treatment did not affect the number of input cells. BenzylGalNAc, tunicamycin and PNGase F had no effect on the number of input cells (data not shown).

**FCV binding and infection is blocked by sialic acid**

We examined the ability of monosaccharides and oligosaccharides to inhibit FCV binding/infection. F9 was incubated with varying concentrations of NANA, NGNA, SL or galactose before binding. As shown in Fig. 7(a),...
concentrations of NANA and NGNA of 40 mM or above completely inhibited binding of F9 to CRFK cells. At 20 mM, NANA and NGNA reduced binding to 32 and 31 %, respectively, of the control and no inhibition was seen at 10 mM. SL and galactose had no effect on virus binding. NANA and NGNA were also able to inhibit infection of CRFK cells by F9 and FCV clinical isolates at the same concentrations used to block binding. Infection with all FCV strains was completely inhibited at 40 mM NANA and NGNA. At 20 mM NANA and NGNA, levels of infection were 21–34 % and 21–31 %, respectively. No inhibition was observed at 10 mM. No inhibition was observed at 10 mM NANA or NGNA or at 160 mM SL or galactose. EIV was also completely inhibited by soluble NANA and NGNA at 40 mM. At 20 mM NANA and NGNA, levels of infection were 48 and 51 %, respectively. No inhibition was observed at 10 mM. Control samples comprised cells incubated in the presence of the saccharides without virus. Concentrations of sugars up to 160 mM were tested. Cell numbers were counted and confirmed that this treatment did not affect the number of input cells (data not shown).

**DISCUSSION**

The earliest events in virus infection involve the interaction of virions with cell-surface molecules. In this study, we
examined the role of sialic acid on cell-surface molecules as a component of the receptor for FCV on CRFK cells.

We studied the interactions of the vaccine strain F9 of FCV and a number of clinical isolates of FCV with cells. We initially examined the ability of FCV to interact with cells from a range of species. We showed that FCV could bind efficiently to cells of feline origin (CRFK and AK-D) and partially to canine cells (MDCK), but that the virus was unable to bind to murine (BHK), simian (Vero) or human (293T) cells.

An earlier study by Kreutz et al. (1994) suggested a role for oligosaccharides in FCV binding to cells. We first sought to verify this observation using NaIO4 treatment of cells. We included a control virus, EIV, which is known to interact with molecules containing z2,3-linked sialic acid residues (Ito et al., 1997). As expected this virus was inhibited by pre-treatment of cells with sialidase S and MAL, but not SNL, confirming the role of z2,3-linked sialic acid in the infection. We were also able to show that the sialic acid moiety could be linked to either a glycolipid or protein, as both PDMP treatment and protease digestion of cells reduced infection by EIV. This has been shown previously for human influenza A and influenza B viruses (Skehel & Wiley, 2000; Chu & Whittaker, 2004).

Sialic acid is used as a receptor by a diverse range of virus families. Many viruses display preferential binding for sialic acid with a particular glycosidic linkage, either z2,3 or z2,6 linkage. Differences in sialic acid linkage can play an important role in virus tissue tropism and pathogenesis. For example, haemagglutinins from avian influenza A viruses recognize z2,3-linked sialic acid found on avian intestinal epithelium, whereas human strains recognize z2,6-linked sialic acid, which is found on human respiratory epithelial cells. Mutations in haemagglutinin are necessary for adaptation of avian strains to bind z2,6-linked sialic acid and thus to grow in the human respiratory tract (Couceiro et al., 1993; Matrosovich et al., 2004).

Murine polyomavirus can bind to an oligosaccharide sequence containing z2,3-linked sialic acid and also to a branched oligosaccharide containing both z2,3- and z2,6-linked sialic acid. The branched sequence is recognized by small plaque-forming, non-tumorigenic strains, whereas large plaque-forming, tumorigenic strains recognize the unbranched sequence. A single amino acid change in VP1 is responsible for the change from small-plaque to large-plaque phenotype and thus determines the different receptor usage (Bauer et al., 1999).

We included a control virus, EIV, which is known to interact with molecules containing z2,3-linked sialic acid residues (Ito et al., 1997). As expected this virus was inhibited by pre-treatment of cells with sialidase S and MAL, but not SNL, confirming the role of z2,3-linked sialic acid in the infection. We were also able to show that the sialic acid moiety could be linked to either a glycolipid or protein, as both PDMP treatment and protease digestion of cells reduced infection by EIV. This has been shown previously for human influenza A and influenza B viruses (Skehel & Wiley, 2000; Chu & Whittaker, 2004).

Two human polyomaviruses, JC virus and BK virus, bind to z2,6- and z2,3-linked sialic acid, respectively (Dugan et al., 2005; Liu et al., 1998). JC virus infection is associated with progressive multifocal leukoencephalopathy. This condition results from JC virus-induced lytic destruction of myelin-producing oligodendrocytes in the brain. BK virus infection of kidneys in renal transplant recipients results in a gradual loss of graft function known as polyomavirus-associated nephropathy. The receptor for JC virus was shown to be an N-linked glycoprotein containing z2,6-linked sialic acid. Cells implicated in JC virus infection, including B cells, oligodendrocytes and astrocytes, have been shown to express higher levels of z2,6-linked compared with z2,3-linked sialic acid, suggesting a correlation between sialic acid linkage and disease.

A number of viruses that display a tropism for ocular tissues show a preference for binding z2,3-linked sialic acid. These include adenovirus type 37, enterovirus 70 and some cases of infection with avian Influenza A virus (Arnb erg et al., 2000; Nokhbeh et al., 2005). However, this binding to z2,3-linked sialic acid is not a prerequisite for ocular tropism, as other
viruses that do not bind sialic acid, such as herpes simplex virus, are also associated with ocular infections.

As mentioned above, Makino et al. (2006) recently identified JAM-A as a functional receptor for FCV. JAM-A is found expressed on cells from a number of different tissues and this broad tissue distribution of JAM-A precludes it from being the only determinant of FCV binding; typically FCV infection results in oral disease and disease of the upper respiratory tract. FCV binding to CRFK cells in vitro is inhibited only partially by the addition of anti-JAM-A antibodies, also supporting the idea that additional components are required. It is likely that the additional binding and tissue specificity is provided by the interaction of FCV with a glycan containing $\alpha_2,6$-linked sialic acid as we have described. It is also possible that the glycan moiety could be associated with JAM-A. The protein contains three potential $N$-linked glycosylation sites; however, the glycosylation state of JAM-A has not been investigated. We are currently examining feline JAM-A for the presence of $N$-linked glycans containing sialic acid.

Highly virulent strains of FCV have emerged recently that are capable of causing systemic febrile disease and death (Coyne et al., 2006; Pedersen et al., 2000). Sequencing of these virulent strains has revealed mutations in the capsid gene that potentially could lead to altered receptor usage (Foley et al., 2006). It is possible that these strains may continue to interact with the broadly expressed JAM-A, resulting in the systemic disease, and that a tissue tropism-determining factor such as a carbohydrate moiety has been altered.

JAM-A is also used as a receptor by prototype and field strains of reoviruses (Barton et al., 2001b; Campbell et al., 2005). Interestingly, these viruses also use carbohydrates as additional receptors and, in common with FCV, in a number of cases, sialic acid is the additional component. An example of this is the interaction of reovirus type 3 with sialic acid, which enhances spread in the host and targets the virus to the bile duct epithelium where it results in biliary disease (Barton et al., 2003).

The data presented here demonstrate that the interaction between FCV and a glycan moiety containing $\alpha_2,6$-linked sialic acid is crucial to FCV infection. It is also likely that this sialic acid-containing glycan, and/or the protein it is linked to, is involved in determining the tissue tropism shown by FCV.

**REFERENCES**


